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RHODANINE DERIVATIVES FOR USE IN A METHOD OF INHIBITING AMYLOID PROTEIN AGGREGATION AND IMAGING AMYLOID DEPOSITS

FIELD OF THE INVENTION

This invention relates to a method of inhibiting amyloid protein aggregation and imaging amyloid deposits. More particularly, this invention relates to a method of inhibiting amyloid protein aggregation, for example to treat Alzheimer's disease using rhodanine derivatives.

BACKGROUND OF THE INVENTION

Amyloidosis is a condition characterized by the accumulation of various insoluble, fibrillar proteins in the tissues of a patient. The fibrillar proteins that comprise the accumulations or deposits are called amyloid proteins. While the particular proteins or peptides found in the deposits vary, the presence of fibrillar morphology and a large amount of β-sheet secondary structure is common to many types of amyloids. An amyloid deposit is formed by the aggregation of amyloid proteins, followed by the further combination of aggregates and/or amyloid proteins.

The presence of amyloid deposits has been shown in various diseases, each with its particular associated protein, such as Mediterranean fever, Muckle-Wells syndrome, idiopathetic myeloma, amyloid polyneuropathy, amyloid cardiomyopathy, systemic senile amyloidosis, amyloid polyneuropathy, hereditary cerebral hemorrhage with amyloidosis, Alzheimer's disease, Down's syndrome, Scrapie, Creutzfeldt-Jacob disease, Kuru, Gerstmann-Straussler-Scheinker syndrome, medullary carcinoma of the thyroid, Isolated atrial amyloid, β_2-microglobulin amyloid in dialysis patients, inclusion body myositis, β_2-amyloid deposits in muscle wasting disease, Sickle Cell Anemia, Parkinson's disease, and Islets of Langerhans diabetes Type 2 insulinoma.

Alzheimer's disease is a degenerative brain disorder characterized clinically by progressive loss of memory, cognition, reasoning, judgement, and emotional stability that gradually leads to mental deterioration and ultimately death. Because Alzheimer's disease and related degenerative brain disorders are a major medical issue for an increasingly aging population, the need for new treatments and methods for diagnosing the disorders are needed.

A simple, noninvasive method for detecting and quantitating amyloid deposits in a patient has been eagerly sought. Presently, detection of amyloid deposits involves histological analysis of biopsy or autopsy materials. Both methods have major drawbacks. For example, an autopsy can only be used for a postmortem diagnosis.

The direct imaging of amyloid deposits in vivo is difficult, as the deposits have many of the same physical properties (ie, density and water content) as normal tissues. Attempts to image amyloid deposits directly using magnetic resonance imaging (MRI) and computer-assisted tomography (CAT) have been disappointing and have detected amyloid deposits only under certain favorable conditions. In addition, efforts to label amyloid deposits with antibodies, serum amyloid P protein, or other probe molecules has provided some selectivity on the periphery of tissues, but has provided for poor imaging of tissue interiors.

Thus, it would be useful to have a noninvasive technique for imaging and quantitating amyloid deposits in a patient. In addition, it would be useful to have compounds that inhibit the aggregation of amyloid proteins to form amyloid deposits.

US Patent Number 5,523,314 relates to rhodanine compounds useful as hypoglycemic agents and for treating Alzheimer's disease. The presently claimed compounds can be distinguished from the compounds disclosed in the patent because the patent does not provide for R^2 to be -$(CH_2)_n$-C_3-C_6 cycloalkyl, -$(CH_2)_m$-phenyl, or for R^1 and R^2 together with the nitrogen atom of Formula I to be a cyclic structure. Moreover, the specific compounds disclosed in the present application show unexpectedly superior activity when compared with the specific compounds disclosed in the 5,523,314 patent. Representative examples in the

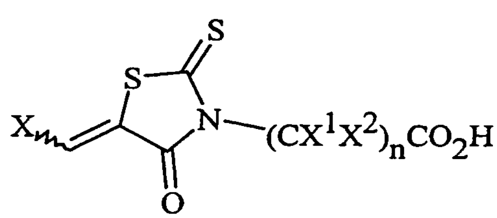
5,523,314 patent were tested in the BASST and BASSR assay set forth below with the following results.

Example No. in Patents	BASST IC_{50} µM	BASSR IC_{50} µM
2	>100	>100
5	100	>100
15	52	>100
28	20	>100
32	>100	>100
35	40	>100
39	>100	>100
42	100	>100
53	>50	>100
77	25	>100
82	100	>100
87	4	>100

Compounds of the present invention comprise both a nitrogen atom substituted with R^1 and R^2 attached to a cyclic or bicyclic group and a $-CO_2H$ group attached to a rhodanine group. None of the compounds exemplified in the 5,523,314 patent contain both functional groups. Additionally, the invention compounds are potent inhibitors of amyloid formation in the above assays.

SUMMARY OF THE INVENTION

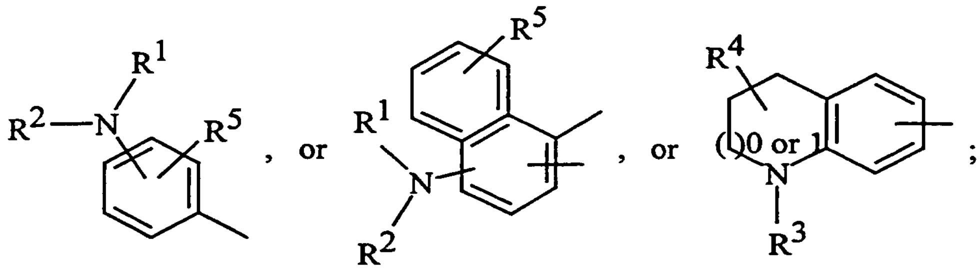
The present invention provides compounds of the Formula I



I

or the pharmaceutically acceptable salts thereof,

wherein X is



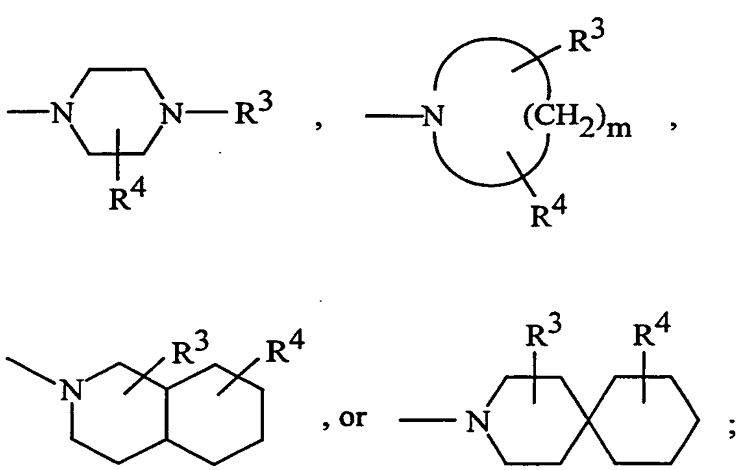
each n is independently 1 to 3 inclusive;

X^1 and X^2 are independently hydrogen or C_1-C_8 alkyl, or $-(CH_2)_y$-Z;

y is 0 to 4 inclusive;

Z is hydrogen, C_1-C_8 alkyl, C_3-C_8 cycloalkyl, C_1-C_8 perfluoroalkyl, C_2-C_8 alkenyl, phenyl, substituted phenyl, naphthyl, substituted naphthyl, -OH, $-OC_1$-C_8 alkyl, $-SC_1$-C_8 alkyl, $-SO_3H$, $-CO_2H$, $-CO_2C_1$-C_8 alkyl, $-\overset{O}{\overset{\|}{C}}HN_2$, $-\overset{O}{\overset{\|}{C}}NH(C_1\text{-}C_8\text{alkyl})$, $-\overset{O}{\overset{\|}{C}}N(C_1\text{-}C_8\text{alkyl})_2$, $-NH_2$, $-NH(C_1\text{-}C_8\text{alkyl})$, $-N(C_1\text{-}C_8\text{alkyl})_2$, $-N\overset{O}{\overset{\|}{C}}C_1\text{-}C_8$ alkyl, guanidinyl, thienyl, imidazolyl, thiazolyl, or indolyl;

R^1 and R^2 are independently C_1-C_8alkyl or $-(CH_2)_n$-C_3-C_6cycloalkyl, $-(CH_2)_n$-phenyl, or R^1 and R^2 taken together with the nitrogen atom to which they are attached form a cyclic structure selected from



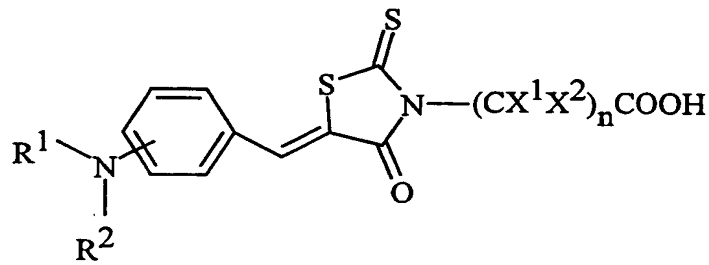
;

where R^3 and R^4 independently are hydrogen, C_1-C_8 alkyl, -$(CH_2)_n$-phenyl, or -$(CH_2)_n$ cycloalkyl;

R^5 is hydrogen, C_1-C_8 alkyl, halogen or -CF_3;

and each m is 2 to 8 inclusive.

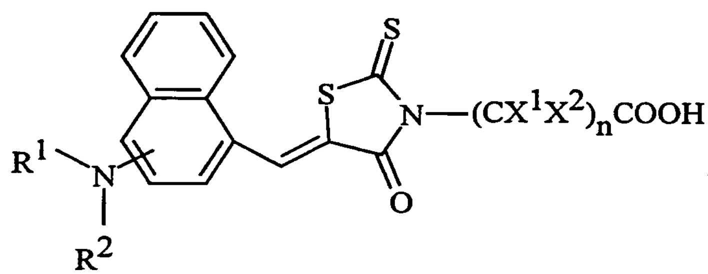
A preferred group of compounds are benzylidene derivatives of Formula II



II

wherein R^1, R^2, X^1, X^2, and n are as defined above.

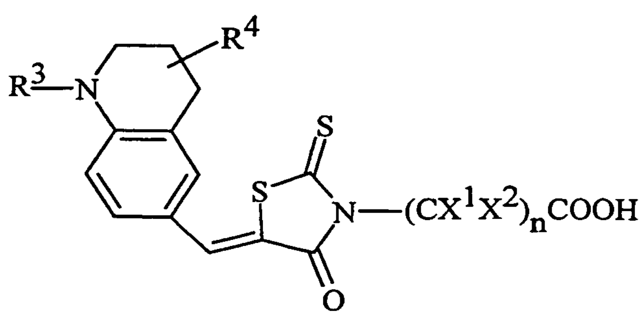
Another preferred group of compounds are naphthalenylmethylene derivatives of Formula III



III

wherein R^1, R^2, X^1, X^2, and n are as defined above.

Still another preferred group of compounds are quinolinylmethylene derivatives of Formula IV



IV

wherein R^3, R^4, X^1, X^2, and n are as defined above.

In a preferred embodiment, the group $-NR^1R^2$ is located at the para position of the aromatic ring portion of X, for example 4-aminophenyl.

Also preferred are compounds of Formula I where X has the Z geometry on the double bond.

Also preferred are compounds of Formula I wherein R^2 is $(CH_2)_n$-C_3-C_6 cycloalkyl or -$(CH_2)_n$ phenyl when R^1 is C_1-C_8 alkyl.

In a most preferred embodiment, the compounds of Formula I are:

(Z) [5-(4-Diethylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid;

(Z) [5-(4-Dibutylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid;

(Z) [5-(4-Dipropylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid;

(Z) [5-(4-Diisobutylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid;

(Z) [5-(4-Dipentylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid;

(Z) (5-{4-[Bis-(3-methyl-butyl)-amino]-benzylidene}-4-oxo-2-thioxo-thiazolidin-3-yl)-acetic acid;

(Z) [5-(4-Azepan-1-yl-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid;

(Z) [5-(4-Dihexylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid;

(Z) {5-[4-(Methyl-octyl-amino)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid;

(Z) {5-[4-(Octahydro-isoquinolin-2-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid;

(Z) {5-[4-(Cyclopropylmethyl-propyl-amino)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid;

(Z) {5-[4-(Hexyl-methyl-amino)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid;

(Z) {5-[4-(Methyl-phenethyl-amino)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid;

(Z) {5-[4-(3-Aza-spiro[5.5]undec-3-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid;

(Z) 3-[5-(4-Dibutylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-propionic acid;

(Z) {5-[4-(Butyl-methyl-amino)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid;

(Z) {5-[4-(Butyl-ethyl-amino)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid;

(Z) {5-[4-(Benzyl-butyl-amino)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid;

(Z) [5-(4-Dioctylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid;

(Z) 4-{5-[4-(Hexyl-methyl-amino)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-butyric acid;

(Z) 3-{5-[4-(Hexyl-methyl-amino)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propionic acid;

(Z) 3-[5-(4-Dipentylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-propionic acid;

(Z) 4-[5-(4-Dibutylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-butyric acid;

(Z) 4-[5-(4-Dipentylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-butyric acid;

(Z) 2-[5-(4-Dibutylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-propionic acid;

(Z) 2-[5-(4-Dibutylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-3-phenyl-propionic acid;

(Z) 2-[5-(4-Dibutylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-3-(3H-imidazol-4-yl)-propionic acid;

(Z) {5-[4-(Hexyl-methyl-amino)-naphthalen-1-ylmethylene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid;

(Z) [4-Oxo-5-(4-pyrrolidin-1-yl-benzylidene)-2-thioxo-thiazolidin-3-yl]-acetic acid;

(Z) {5-[4-(4-Butyl-piperazin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid;

(Z) (4-Oxo-5-{4-[4-(3-phenyl-propyl)-piperidin-1-yl]-benzylidene}-2-thioxo-thiazolidin-3-yl)-acetic acid;

(Z) {5-[4-(Octahydro-isoquinolin-2-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid;

(Z) 3-{5-[4-(3-Aza-spiro[5.5]undec-3-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propionic acid;

(Z) 3-[4-Oxo-5-(4-azepan-1-yl-benzylidene)-2-thioxo-thiazolidin-3-yl]-propionic acid;

(Z) 4-{5-[4-(3-Aza-spiro[5.5]undec-3-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-butyric acid;

(Z) {4-Oxo-5-[4-(4-propyl-piperidin-1-yl)-benzylidene]-2-thioxo-thiazolidin-3-yl}-acetic acid;

(Z) 3-{4-Oxo-5-[4-(4-propyl-piperidin-1-yl)-benzylidene]-2-thioxo-thiazolidin-3-yl}-propionic acid;

(Z) 4-{4-Oxo-5-[4-(4-propyl-piperidin-1-yl)-benzylidene]-2-thioxo-thiazolidin-3-yl}-butyric acid;

(Z) [5-(1-Butyl-1,2,3,4-tetrahydro-quinolin-6-ylmethylene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid;

(Z) 3-{5-[(4aS,8aR)-4-(Octahydro-isoquinolin-2-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propionic acid;

(Z) 4-{5-[(4aS,8aR)-4-(Octahydro-isoquinolin-2-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-butyric acid;

(Z) [4-Oxo-5-(4-piperidin-1-yl-benzylidene)-2-thioxo-thiazolidin-3-yl]-acetic acid;

(Z) 3-{5-[(4aS,8aS)-4-(Octahydro-isoquinolin-2-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propionic acid;

(Z) 4-[4-Oxo-5-(4-azepan-1-yl-benzylidene)-2-thioxo-thiazolidin-3-yl]-butyric acid;

(Z) 4-{5-[(4aS,8aS)-4-(Octahydro-isoquinolin-2-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-butyric acid;

(Z) 3-[4-Oxo-5-(4-piperidin-1-yl-benzylidene)-2-thioxo-thiazolidin-3-yl]-propionic acid;

(Z) 4-[4-Oxo-5-(4-piperidin-1-yl-benzylidene)-2-thioxo-thiazolidin-3-yl]-butyric acid;

(Z) [4-Oxo-5-(4-azepan-1-yl-benzylidene)-2-thioxo-thiazolidin-3-yl]-acetic acid;

(Z) {5-[4-(4-Ethyl-4-methyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid;

(Z) 3-{5-[4-(4-Ethyl-4-methyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propionic acid;

(Z) {5-[4-(4-Cyclohexylmethyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid;

(Z) [5-(1-Butyl-2,3-dihydro-1H-indol-5-ylmethylene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid;

(Z) 4-{5-[4-(4-Ethyl-4-methyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-butyric acid;

(Z) 3-{5-[4-(4-Cyclohexylmethyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propionic acid;

(Z) 3-{5-[4-(4-Benzyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propionic acid;

(Z) {5-[4-(4-Benzyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid;

(Z) 4-[4-Oxo-5-(4-azocan-1-yl-benzylidene)-2-thioxo-thiazolidin-3-yl]-butyric acid;

(Z) 4-{5-[4-(4-Benzyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-butyric acid;

(Z) 4-{5-[4-(4-Cyclohexylmethyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-butyric acid;

(Z) 3-[4-Oxo-5-(4-azocan-1-yl-benzylidene)-2-thioxo-thiazolidin-3-yl]-propionic acid;

(Z) 3-[5-(1-Butyl-1,2,3,4-tetrahydro-quinolin-6-yl-methylene)-4-oxo-2-thioxo-thiazolidin-3-yl]-propionic acid;

(Z) 4-[5-(1-Butyl-1,2,3,4-tetrahydro-quinolin-6-yl-methylene)-4-oxo-2-thioxo-thiazolidin-3-yl]-butyric acid;

(Z) {5-[4-(4-Hexyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid;

(Z) 3-{5-[4-(4-Hexyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propionic acid;

(Z) 4-{5-[4-(4-Hexyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-butyric acid;

(Z) {5-[4-(4-Butyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid;

(Z) 3-{5-[4-(4-Butyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propionic acid;

(Z) 4-{5-[4-(4-Butyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-butyric acid;

(Z) {5-[4-(4-Pentyl-pyrrolidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid;

(Z) 3-{5-[4-(4-Pentyl-pyrrolidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propionic acid;

(Z) 4-{5-[4-(4-Pentyl-pyrrolidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-butyric acid.

Also provided is a method of treating Alzheimer's disease, the method comprising administering to a patient having Alzheimer's disease a therapeutically effective amount of a compound of Formula I

The invention also provides pharmaceutical formulations comprising a compound of Formula I admixed with a pharmaceutically acceptable diluent, excipient, or carrier therefor.

Also provided is a method of inhibiting the aggregation of amyloid proteins to form amyloid deposits, the method comprising administering to a patient in need of inhibition of amyloid protein aggregation an amyloid protein aggregation inhibiting amount of a compound of Formula I.

Also provided is a method of imaging amyloid deposits, the method comprising the steps of:

a. introducing into a patient a detectable quantity of a labeled compound of Formula I

b. allowing sufficient time for the labeled compound to become associated with amyloid deposits; and

c. detecting the labeled compound associated with the amyloid deposits.

In a preferred embodiment of the method, the patient has or is suspected to have Alzheimer's disease.

In another preferred embodiment, the labeled compound is a radiolabled compound.

In another preferred embodiment, the labeled compound is detected using MRI.

Also provided is a pharmaceutical composition comprising a compound of Formula I.

DETAILED DESCRIPTION OF THE INVENTION

The term "alkyl" means a straight or branched chain hydrocarbon. Representative examples of alkyl groups are methyl, ethyl, propyl, isopropyl, isobutyl, butyl, tert-butyl, sec-butyl, pentyl, and hexyl.

Preferred alkyl groups are C_1-C_8 alkyl.

The term "alkoxy" means an alkyl group such as C_1-C_8 alkyl attached to an oxygen atom. Representative examples of alkoxy groups include methoxy, ethoxy, tert-butoxy, propoxy, and isobutoxy.

The term "halogen" includes chlorine, fluorine, bromine, and iodine.

The term "substituted" means that one or more hydrogen atom in a molecule has been replaced with another atom or group of atoms. For example, substituents include halogen, -OH, $-CF_3$, $-NO_2$, $-NH_2$, $-NH(C_1$-C_6alkyl), $-N(C_1$-C_6alkyl$)_2$, C_1-C_6 alkyl, $-OC_1$-C_6 alkyl, -CN, $-CF_3$, $-CO_2H$, and $-CO_2C_1$-C_6 alkyl.

The term “substituted phenyl” means a phenyl ring in which from 1 to 4 hydrogen atoms have been independently replaced with a substituent, preferably one selected from the list above.

The symbol “-” means a covalent bond.

The term “pharmaceutically acceptable salt, ester, amide, and prodrug” as used herein refers to those carboxylate salts, amino acid addition salts, esters, amides, and prodrugs of the compounds of the present invention which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of patients without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the invention. The term “salts” refers to the relatively nontoxic, inorganic and organic acid addition salts of compounds of the present invention. These salts can be prepared in situ during the final isolation and purification of the compounds or by separately reacting the purified compound in its free base form with a suitable organic or inorganic acid and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, nitrate, acetate, oxalate, valerate, oleate, palmitate, stearate, laurate, borate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate mesylate, glucoheptonate, lactiobionate and laurylsulphonate salts, and the like. These may include cations based on the alkali and alkaline earth metals, such as sodium, lithium, potassium, calcium, magnesium, and the like, as well as, nontoxic ammonium, quaternary ammonium and amine cations including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. (See, for example, Berge S.M., et al., Pharmaceutical Salts, *J. Pharm. Sci.*, 66:1-19 (1977) which is incorporated herein by reference.)

Examples of pharmaceutically acceptable, nontoxic esters of the compounds of this invention include C_1-C_6 alkyl esters wherein the alkyl group is a straight or branched chain. Acceptable esters also include C_5-C_7 cycloalkyl esters as well as arylalkyl esters such as, but not limited to benzyl. C_1-C_4 alkyl

esters are preferred. Esters of the compounds of the present invention may be prepared according to conventional methods.

Examples of pharmaceutically acceptable, nontoxic amides of the compounds of this invention include amides derived from ammonia, primary C_1-C_6 alkyl amines and secondary C_1-C_6 dialkyl amines wherein the alkyl groups are straight or branched chain. In the case of secondary amines, the amine may also be in the form of a 5- or 6-membered heterocycle containing one nitrogen atom. Amides derived from ammonia, C_1-C_3 alkyl primary amides and C_1-C_2 dialkyl secondary amides are preferred. Amides of the compounds of the invention may be prepared according to conventional methods.

The term "prodrug" refers to compounds that are rapidly transformed in vivo to yield the parent compound of the above formulas, for example, by hydrolysis in blood. A thorough discussion is provided in T. Higuchi and V. Stella, Pro-drugs as Novel Delivery Systems, Vol. 14 of the A.C.S. Symposium Series, and in Bioreversible Carriers in Drug Design, ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987, both of which are incorporated herein by reference.

In addition, the compounds of the present invention can exist in unsolvated as well as solvated forms with pharmaceutically acceptable solvents such as water, ethanol, and the like. In general, the solvated forms are considered equivalent to the unsolvated forms for the purposes of the present invention.

The compounds of the present invention can exist in different stereoisometric forms by virtue of the presence of asymmetric centers in the compounds. It is contemplated that all stereoisometric forms of the compounds, as well as mixture thereof, including racemic mixtures, form part of this invention.

In the first step of the present method of imaging, a labeled compound of Formula I is introduced into a tissue or a patient in a detectable quantity. The compound is typically part of a pharmaceutical composition and is administered to the tissue or the patient by methods well-known to those skilled in the art.

In the methods of the present invention, a compound can be administered either orally, rectally, parenterally (intravenous, by intramuscularly or

subcutaneously), intracisternally, intravaginally, intraperitoneally, intravesically, locally (powders, ointments or drops), or as a buccal or nasal spray.

Compositions suitable for parenteral injection may comprise physiologically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, and sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents, or vehicles include water, ethanol, polyols (propyleneglycol, polyethyleneglycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil), and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants.

These compositions may also contain adjuvants such as preserving, wetting, emulsifying, and dispensing agents. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, for example sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is admixed with at least one inert customary excipient (or carrier) such as sodium citrate or dicalcium phosphate or (a) fillers or extenders, as for example, starches, lactose, sucrose, glucose, mannitol, and silicic acid; (b) binders, as for example, carboxymethylcellulose, alignates, gelatin, polyvinylpyrrolidone, sucrose, and acacia; (c) humectants, as for example, glycerol; (d) disintegrating agents, as for example, agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain complex silicates and sodium carbonate; (e) solution retarders, as for example paraffin; (f) absorption accelerators, as for example, quaternary ammonium compounds; (g) wetting agents, as for example, cetyl alcohol and glycerol monostearate; (h) adsorbents, as for example, kaolin and bentonite; and (i) lubricants, as for example, talc, calcium stearate, magnesium stearate, solid

polyethylene glycols, sodium lauryl sulfate, or mixtures thereof. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft- and hard-filled gelatin capsules using such excipients as lactose or milk sugar, as well as high molecular weight polyethyleneglycols, and the like.

Solid dosage forms such as tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells, such as enteric coatings and others well known in the art. They may contain opacifying agents, and can also be of such composition that they release the active compound or compounds in a certain part of the intestinal tract in a delayed manner. Examples of embedding compositions which can be used are polymeric substances and waxes. The active compounds can also be in microencapsulated form, if appropriate, with one or more of the above-mentioned excipients.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art, such as water or other solvents, solubilizing agents and emulsifiers, as for example, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propyleneglycol, 1,3-butyleneglycol, dimethylformamide, oils, in particular, cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil, and sesame oil, glycerol, tetrahydrofurfuryl alcohol, polyethyleneglycols, and fatty acid esters of sorbitan or mixtures of these substances, and the like.

Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Suspensions, in addition to the active compounds, may contain suspending agents, as for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, or mixtures of these substances, and the like.

Compositions for rectal administrations are preferably suppositories which can be prepared by mixing the compounds of the present invention with suitable nonirritating excipients or carriers such as cocoa butter, polyethyleneglycol or a

suppository wax, which are solid at ordinary temperatures but liquid at body temperature and therefore, melt in the rectum or vaginal cavity and release the active component.

Dosage forms for topical administration of a compound of this invention include ointments, powders, sprays, and inhalants. The active component is admixed under sterile conditions with a physiologically acceptable carrier and any preservatives, buffers or propellants as may be required. Ophthalmic formulations, eye ointments, powders, and solutions are also contemplated as being within the scope of this invention.

In a preferred embodiment of the invention, the labeled compound is introduced into a patient in a detectable quantity and after sufficient time has passed for the compound to become associated with amyloid deposits, the labeled compound is detected noninvasively inside the patient. In another embodiment of the invention, a labeled compound of Formula I is introduced into a patient, sufficient time is allowed for the compound to become associated with amyloid deposits, and then a sample of tissue from the patient is removed and the labeled compound in the tissue is detected apart from the patient. In a third embodiment of the invention, a tissue sample is removed from a patient and a labeled compound of Formula I is introduced into the tissue sample. After a sufficient amount of time for the compound to become bound to amyloid deposits, the compound is detected.

The administration of the labeled compound to a patient can be by a general or local administration route. For example, the labeled compound may be administered to the patient such that it is delivered throughout the body. Alternatively, the labeled compound can be administered to a specific organ or tissue of interest. For example, it is desirable to locate and quantitate amyloid deposits in the brain in order to diagnose or track the progress of Alzheimer's disease in a patient.

The term "tissue" means a part of a patient's body. Examples of tissues include the brain, heart, liver, blood vessels, and arteries. A detectable quantity is a quantity of labeled compound necessary to be detected by the detection method chosen. The amount of a labeled compound to be introduced into a patient in order to provide for detection can readily be determined by those skilled in the art. For

example, increasing amounts of the labeled compound can be given to a patient until the compound is detected by the detection method of choice. A label is introduced into the compounds to provide for detection of the compounds.

The term "patient" means humans and other animals. Those skilled in the art are also familiar with determining the amount of time sufficient for a compound to become associated with amyloid deposits. The amount of time necessary can easily be determined by introducing a detectable amount of a labeled compound of Formula I into a patient and then detecting the labeled compound at various times after administration.

The term "associated" means a chemical interaction between the labeled compound and the amyloid deposit. Examples of associations include covalent bonds, ionic bonds, hydrophilic-hydrophilic interactions, hydrophobic-hydrophobic interactions, and complexes.

Those skilled in the art are familiar with the various ways to detect labeled compounds. For example, magnetic resonance imaging (MRI), positron emission tomography (PET), or single photon emission computed tomography (SPECT) can be used to detect radiolabeled compounds. The label that is introduced into the compound will depend on the detection method desired. For example, if PET is selected as a detection method, the compound must possess a positron-emitting atom, such as ^{11}C or ^{18}F.

Another example of a suitable label in a compound of Formula I is an atom such as ^{13}C, ^{15}N, or ^{19}F which can be detected using magnetic resonance imaging (MRI) which is also sometimes called nuclear magnetic resonance (NMR). In addition, the labeled compounds of Formula I may also be detected by MRI using paramagnetic contrast agents.

Another example of detection is electron paramagnetic resonance (EPR). In this case, EPR probes which are well-known in the art, such as nitroxides, can be used.

The imaging of amyloid deposits can also be carried out quantitatively so that the amount of amyloid deposits can be determined.

The present invention also provides a method of inhibiting the aggregation of amyloid proteins to form amyloid deposits, by administering to a patient in

need of inhibition of the aggregation of amyloid protein an amyloid protein inhibiting amount of a compound of Formula I. Those skilled in the art are readily able to determine an amyloid inhibiting amount by simply administering a compound of Formula I to a patient in increasing amounts until the growth of amyloid deposits is decreased or stopped. The rate of growth can be assessed using imaging or by taking a tissue sample from a patient and observing the amyloid deposits therein.

A patient in need of inhibition of the aggregation of amyloid proteins is a patient having a disease or condition in which amyloid proteins aggregate. Examples of such diseases and conditions include Mediterranean fever, Muckle-Wells syndrome, idiopathetic myeloma, amyloid polyneuropathy, amyloid cardiomyopathy, systemic senile amyloidosis, amyloid polyneuropathy, hereditary cerebral hemorrhage with amyloidosis, Alzheimer's disease, Down's syndrome, Scrapie, Creutzfeldt-Jacob disease, Kuru, Gerstmann-Straussler-Scheinker syndrome, medullary carcinoma of the thyroid, Isolated atrial amyloid, β_2-microglobulin amyloid in dialysis patients, inclusion body myositis, β_2-amyloid deposits in muscle wasting disease, Sickle Cell Anemia, Parkinson's disease, and Islets of Langerhans diabetes Type 2 insulinoma.

Also provided by the present invention are compounds of Formula I wherein one or more atom in the compound has been replaced with a radioisotope. The radioisotope can be any radioisotope. However,^{3}H, ^{123}I, ^{1}I, ^{131}I, ^{11}C, and ^{18}F are preferred. Those skilled in the art are familiar with the procedure used to introduce a radioisotope into a compound. For example, compounds of Formula I wherein the COOH group is replaced by ^{11}COOH are readily prepared.

The compounds of the present invention can be administered to a patient at dosage levels in the range of about 0.1 to about 1,000 mg per day, which are "effective amounts" for inhibiting amyloid formation and treating the above mentioned diseases. For a normal human adult having a body weight of about 70 kg, a dosage in the range of about 0.01 to about 100 mg per kilogram of body weight per day is sufficient. The specific dosage used, however, can vary. For example, the dosage can depend on a number of factors including the requirements of the patient, the severity of the condition being treated, and the

pharmacological activity of the compound being used. The determination of optimum dosages for a particular patient is well-known to those skilled in the art.

The examples presented below are intended to illustrate particular embodiments of the invention and are not intended to limit the scope of the specification, including the claims, in any manner.

EXAMPLES

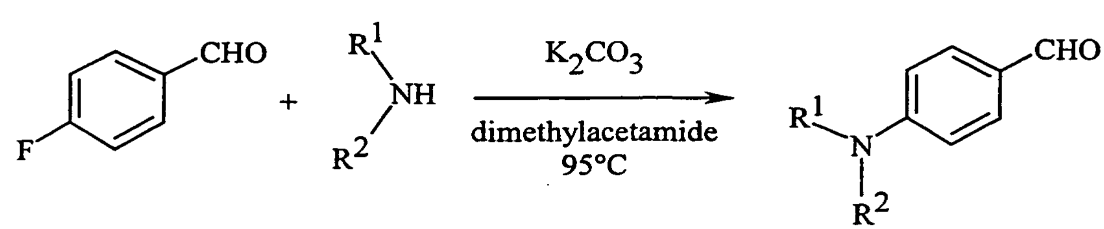
General Synthetic Scheme

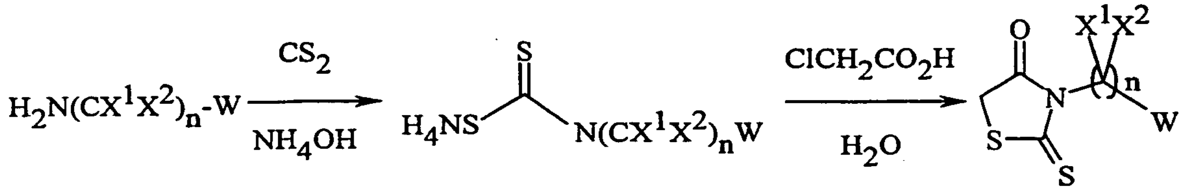
The compounds of Formula I can be prepared by any of several processes, utilizing readily available starting materials and methods well-known in organic chemistry. Scheme I below illustrates a typical method for making starting materials and the final products of Formula I. The invention compounds are generally prepared by reacting a substituted rhodanine with an aldehyde such as benzaldehyde.

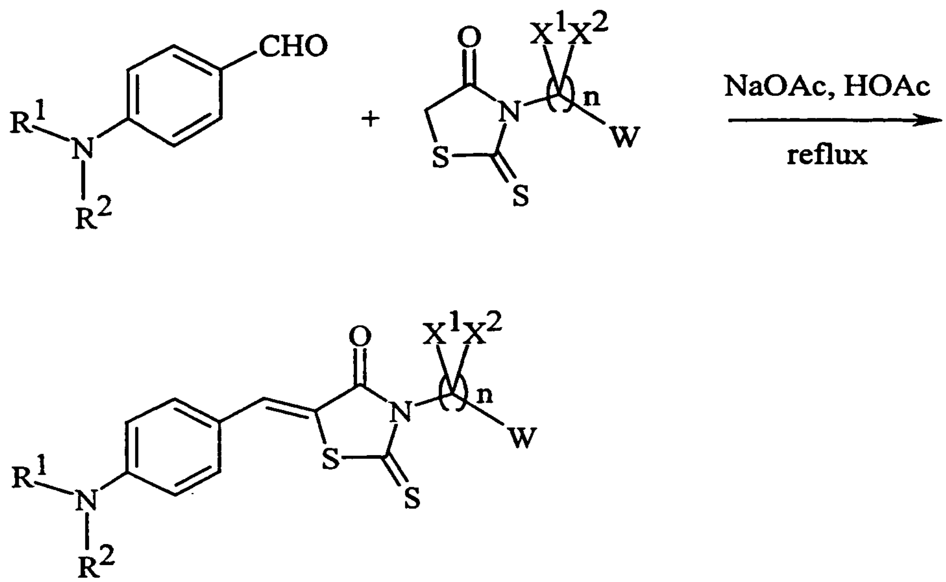
Appropriately substituted amino benzaldehydes are prepared by reacting 4-fluorobenzaldehyde with an amine (HNR^1R^2) in the presence of a base such as potassium carbonate or sodium hydroxide. The reaction generally is carried out in a solvent such as dimethylacetamide or dimethylformamide. Many of the aryl aldehydes are commercially available.

N-substituted rhodanines also are commercially available, or they can be prepared by condensing carbon disulfide and chloroacetic acid with the appropriate amine. The compounds of the present invention can be prepared by condensation of an appropriately N-substituted rhodanine with an appropriately substituted aromatic aldehyde in refluxing glacial acetic acid in the presence of sodium acetate.

Scheme 1







W is COOH or an ester group such as CO_2CH_2Ph.

Example 1

(Z) [5-(4-Diethylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid

Diethylaminobenzaldehyde (1.02 g, 5.75 mmol), rhodanine-3-acetic acid (1.0 g, 5.22 mmol) and sodium acetate (1.28 g, 15.6 mmol) in acetic acid (10 mL) are heated to reflux for 15 hours with stirring. The reaction mixture is cooled, and the precipitated solids are collected by filtration, washed with water and dried under vacuum to provide 1.10 g of the title compound as a red solid; melting point (mp) 240°C. Elemental analysis calculated for $C_{16}H_{18}N_2O_3S_2$: Calculated: C, 54.84; H, 5.18; N, 7.99. Found: C, 54.95; H, 5.09; N, 7.84.

Example 2

(Z) [5-(4-Dibutylamino-benzylidene)-4-oxo-2-thi xo-thiazolidin-3-yl]-acetic acid

Example 2 was prepared according to Example 1, except that dibutylaminobenzaldehyde is substituted for diethylaminobenzaldehyde; mp 268-269°C. Elemental analysis calculated for $C_{20}H_{26}N_2O_3S_2 \cdot 0.25\ H_2O$: Calculated: C, 58.44; H, 6.50; N, 6.82. Found: C, 58.04; H, 6.40; N, 6.44.

Example 3

(Z) [5-(4-Dipropylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid

Step A:

Dipropylamine (10.9 mL, 80.0 mmol), 4-fluorobenzaldehyde (4.2 mL, 40.0 mmol), and potassium carbonate (6.62 g, 48.0 mmol) in dimethylacetamide (20 mL) are heated to 95°C for 3 days with vigorous stirring. The reaction mixture is cooled, diluted with water (200 mL), and extracted with diethyl ether. The organic extract is dried (magnesium sulfate) and concentrated in vacuo. The resulting oil is purified by medium pressure liquid chromatography (MPLC) on silica gel eluting with 5% ethyl acetate/hexane to give 1.42 g of dipropylaminobenzaldehyde as a yellow oil.

Step B:

Example 3 was prepared according to Example 1, except that dipropylaminobenzaldehyde is substituted for diethylaminobenzaldehyde; mp 233°C. Elemental analysis calculated for $C_{18}H_{22}N_2O_3S_2$: Calculated: C, 57.12; H, 5.86; N, 7.40. Found: C, 57.10; H, 5.79; N, 7.39.

Example 4

(Z) [5-(4-Diisobutylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid

Example 4 was prepared according to Example 3, except that diisobutylamine is substituted for dipropylamine in Step A; mp 234°C. Elemental analysis calculated for $C_{20}H_{26}N_2O_3S_2 \cdot 0.1\ H_20$: Calculated: C, 58.82; H, 6.75; N, 6.86. Found: C, 58.43; H, 6.25; N, 6.57.

Example 5

(Z) [5-(4-Dipentylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid

Example 5 was prepared according to Example 3, except that dipentylamine is substituted for dipropylamine in Step A; mp 195-196°C. Elemental analysis calculated for $C_{22}H_{30}N_2O_3S_2$: Calculated: C, 60.80; H, 6.96; N, 6.45. Found: C, 60.60; H, 6.82; N, 6.30.

Example 6

(Z) (5-{4-[Bis-(3-methyl-butyl)-amino]-benzylidene}-4-oxo-2-thioxo-thiazolidin-3-yl)-acetic acid

Example 6 was prepared according to Example 3, except that diisoamylamine is substituted for dipropylamine in Step A; mp 257°C. Elemental analysis calculated for $C_{22}H_{30}N_2O_3S_2 \cdot 0.15\ H_2O$: Calculated: C, 60.42; H, 6.98; N, 6.41. Found: C, 60.06; H, 6.83; N, 6.23.

Example 7

(Z) [5-(4-Azepan-1-yl-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid

Example 7 was prepared according to Example 3, except that hexamethyleneimine is substituted for dipropylamine in Step A; mp 275°C. Elemental analysis calculated for $C_{18}H_{20}N_2O_3S_2$: Calculated: C, 57.42; H, 5.35; N, 7.44. Found: C, 57.25; H, 5.29; N, 7.37.

Example 8

(Z) [5-(4-Dihexylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid

Example 8 was prepared according to Example 3, except that dihexylamine is substituted for dipropylamine in Step A; mp 152-153°C. Elemental analysis calculated for $C_{24}H_{34}N_2O_3S_2$: Calculated: C, 62.30; H, 7.41; N, 6.05. Found: C, 62.05; H, 7.31; N, 5.87.

Example 9

(Z) {5-[4-(Methyl-octyl-amino)-benzylidene]-4-oxo-2-thiox -thiazolidin-3-yl}-acetic acid

Example 9 was prepared according to Example 3, except that N-methyl-N-octylamine is substituted for dipropylamine in Step A; mp 203-204°C. Elemental analysis calculated for $C_{21}H_{28}N_2O_3S_2$: Calculated: C, 59.97; H, 6.71; N, 6.66. Found: C, 59.90; H, 6.62; N, 6.50.

Example 10

(Z) {5-[4-(Octahydro-isoquinolin-2-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid

Example 10 was prepared according to Example 3, except that perhydroisoquinoline is substituted for dipropylamine in Step A; mp 234°C. Elemental analysis calculated for $C_{21}H_{24}N_2O_3S_2$: Calculated: C, 60.55; H, 5.81; N, 6.72. Found: C, 60.40; H, 5.82; N, 6.60.

Example 11

(Z) {5-[4-(Cyclopropylmethyl-propyl-amino)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid

Example 11 was prepared according to Example 3, except that N-propylcyclopropanemethylamine is substituted for dipropylamine in Step A; mp 289°C. Elemental analysis calculated for $C_{19}H_{22}N_2O_3S_2$: Calculated: C, 58.44; H, 5.68; N, 7.17. Found: C, 56.65; H, 5.46; N, 6.92.

Example 12

(Z) {5-[4-(Hexyl-methyl-amino)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid

Example 12 was prepared according to Example 3, except that N-methylhexylamine is substituted for dipropylamine in Step A; mp 207-208°C. Elemental analysis calculated for $C_{19}H_{24}N_2O_3S_2$: Calculated: C, 58.14; H, 6.16; N, 7.14. Found: C, 58.42; H, 6.26; N, 6.89.

Example 13

(Z) {5-[4-(Methyl-phenethyl-amino)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid

Example 13 was prepared according to Example 3, except that N-methylphenethylamine is substituted for dipropylamine in Step A; mp 263-264°C. Elemental analysis calculated for $C_{21}H_{20}N_2O_3S_2$: Calculated: C, 61.14; H, 4.89; N, 6.79. Found: C, 61.12; H, 4.93; N, 6.68

Example 14

(Z) {5-[4-(3-Aza-spiro[5.5]undec-3-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid

Example 14 was prepared according to Example 3, except that 3-azaspiro(5.5)undecane is substituted for dipropylamine in Step A; mp 265°C. Elemental analysis calculated for $C_{22}H_{26}N_2O_3S_2$: Calculated: C, 61.37; H, 6.09; N, 6.51. Found: C, 61.37; H, 6.13; N, 6.42.

Example 15

(Z) 3-[5-(4-Dibutylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-propionic acid

Example 15 was prepared according to Example 2, except that N-carboxyethylrhodanine is substituted for rhodanine-3-acetic acid; mp 164-165°C. Elemental analysis calculated for $C_{21}H_{28}N_2O_3S_2$: Calculated: C, 59.97; H, 6.71; N, 6.66. Found: C, 58.84; H, 6.38; N, 6.61.

Example 16

(Z) {5-[4-(Butyl-methyl-amino)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid

Example 16 was prepared according to Example 3, except that N-methylbutylamine is substituted for dipropylamine in Step A; mp 223°C. Elemental analysis calculated for $C_{17}H_{20}N_2O_3S_2$: Calculated: C, 56.02; H, 5.53; N, 7.69. Found: C, 56.33; H, 5.56; N, 7.66.

Example 17

(Z) {5-[4-(Butyl-ethyl-amino)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid

Example 17 was prepared according to Example 3, except that N-ethylbutylamine is substituted for dipropylamine in Step A; mp 206°C. Elemental analysis calculated for $C_{18}H_{22}N_2O_3S_2$: Calculated: C, 57.12; H, 5.86; N, 7.40. Found: C, 57.24; H, 5.84; N, 7.37.

Example 18

(Z) {5-[4-(Benzyl-butyl-amino)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid

Example 18 was prepared according to Example 3, except that N-benzylbutylamine is substituted for dipropylamine in Step A; mp 205°C. Elemental analysis calculated for $C_{23}H_{24}N_2O_3S_2$: Calculated: C, 62.70; H, 5.49; N, 6.36. Found: C, 63.10; H, 5.51; N, 6.28.

Example 19

(Z) [5-(4-Dioctylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid

Example 19 was prepared according to Example 3, except that dioctylamine is substituted for dipropylamine in Step A; mp 140-145°C. Elemental analysis calculated for $C_{28}H_{42}N_2O_3S_2$: Calculated: C, 64.83; H, 8.16; N, 5.40. Found: C, 64.51; H, 8.17; N, 5.28.

Example 20

(Z) 4-{5-[4-(Hexyl-methyl-amino)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-butyric acid

Step A:

To a mixture of carbon disulfide (10.9 mL, 0.18 mol) and ammonium hydroxide (40 mL) at 0°C is added ethyl-4-aminobutyrate (24.2 g, 0.14 mol). The reaction mixture is warmed to room temperature and stirred for 18 hours. The resulting dithiocarbamate is collected on a filter, washed with a small amount of

diethyl ether, and dried. This dithiocarbamate (10.75 g, 0.048 mol) is added slowly to a cold (0°C) solution of sodium chloroacetate (5.82 g, 0.050 mol) in water (10 mL) made basic with sodium carbonate. The reaction mixture is warmed to room temperature and poured into a warm (70°C) HCl solution (80 mL, 7 M) and heated to 90°C for 1.5 hours. The reaction mixture is cooled, the product collected on a filter, washed with water, and dried to give 8.08 g of rhodanine-3-butyric acid.

Step B:

Example 20 was prepared according to Example 12, except that rhodanine-3-butyric acid is substituted for rhodanine-3-acetic acid; mp 147-150°C. Elemental analysis calculated for $C_{21}H_{28}N_2O_3S_2$: Calculated: C, 59.97; H, 6.71; N, 6.66. Found: C, 57.98; H, 6.45; N, 6.38.

Example 21

(Z) 3-{5-[4-(Hexyl-methyl-amino)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propionic acid

Example 21 was prepared according to Example 1 except that 4-(*n*-hexylmethylamino)benzaldehyde and N-carboxyethylrhodanine are used; mp 169-173°C. Elemental analysis calculated for $C_{20}H_{26}N_2O_3S_2 \cdot 0.21\ H_2O$: Calculated: C, 58.54; H, 6.49; N, 6.83. Found: C, 58.55; H, 6.44; N, 6.71.

Example 22

(Z) 3-[5-(4-Dipentylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-propionic acid

Example 22 was prepared according to Example 1 except 4-(dipentylamino)benzaldehyde and N-carboxyethylrhodanine are used; mp 127-130°C. Elemental analysis calculated for $C_{23}H_{32}N_2O_3S_2 \cdot 0.27\ H_2O$: Calculated: C, 60.91; H, 7.23; N, 6.18. Found: C, 60.91; H, 7.20; N, 6.09.

Example 23

(Z) 4-[5-(4-Dibutylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-butyric acid

Example 23 was prepared according to Example 20 except 4-(dibutylamino)benzaldehyde is substituted for 4-(*n*-hexylmethylamino)-benzaldehyde; mp 145-148°C. Elemental analysis calculated for $C_{22}H_{30}N_2O_3S_2$: Calculated: C, 60.80; H, 6.96; N, 6.45. Found: C, 60.59; H, 6.88; N, 6.33.

Example 24

(Z) 4-[5-(4-Dipentylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-butyric acid

Example 24 was prepared according to Example 20 except 4-(dipentylamino)benzaldehyde is substituted for 4-(*n*-hexylmethylamino)-benzaldehyde; mp 132-133°C. Elemental analysis calculated for $C_{24}H_{34}N_2O_3S_2$: Calculated: C, 62.30; H, 7.41; N, 6.05. Found: C, 62.10; H, 7.28; N, 5.85.

Example 25

(Z) 2-[5-(4-Dibutylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-propionic acid

Example 25 was prepared according to Example 20 except dl-alanine is substituted for ethyl-4-aminobutyrate in Step A and 4-(dibutylamino)-benzaldehyde is substituted for 4-(*n*-hexylmethylamino)benzaldehyde in Step B; mp 168-169°C. Elemental analysis calculated for $C_{21}H_{28}N_2O_3S_2$: Calculated: C, 59.97; H, 6.71; N, 6.66. Found: C, 60.11; H, 6.93; N, 6.76.

Example 26

(Z) 2-[5-(4-Dibutylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-3-phenyl-propionic acid

Example 26 was prepared according to Example 20 except dl-phenylalanine is substituted for ethy-4-aminobutyrate in Step A and 4-(dibutylamino)benzaldehyde is substituted for 4-(*n*-hexylmethylamino)-benzaldehyde in Step B; mp 205°C. Elemental analysis calculated for

$C_{27}H_{32}N_2O_3S_2 \cdot 1.0C_2H_4O_2$: Calculated: C, 62.56; H, 6.52; N, 5.03. Found: C, 62.72; H, 6.69; N, 5.11.

Example 27

(Z) 2-[5-(4-Dibutylamino-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-3-(3H-imidazol-4-yl)-propionic acid

Example 27 was prepared according to Example 20 except dl-histidine is substituted for ethyl-4-aminobutyrate in Step A and 4-(dibutylamino)-benzaldehyde is substituted for 4-(*n*-hexylmethylamino)benzaldehyde in Step B; mp 280-281°C. Elemental analysis calculated for $C_{24}H_{30}N_4O_3S_2$: Calculated: C, 59.23; H, 6.21; N, 11.51. Found: C, 59.02; H, 6.09; N, 11.30.

Example 28

(Z) {5-[4-(Hexyl-methyl-amino)-naphthalen-1-ylmethylene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid

Step A:

N-Methylhexylamine (6.5 mL, 43.0 mmol), 4-fluoro-1-naphthaldehyde (5.0 g, 28.7 mmol, prepared according to *J. Org. Chem.* 1995;60:6592-6594) and potassium carbonate (4.75 g, 34.4 mmol) in dimethylacetamide (10 mL) are heated to 95°C for 3 days with vigorous stirring. The reaction mixture is cooled, diluted with water (100 mL) and extracted with diethyl ether. The organic extract is dried (magnesium sulfate) and concentrated in vacuo. The resulting oil is purified by medium pressure liquid chromatography (MPLC) on silica gel eluting with 5% ethyl acetate/hexane to give 5.94 g of 4-(*n*-hexylmethylamino)-1-naphthaldehyde as a yellow oil.

Step B:

Example 28 was prepared according to Example 1 except that 4-(*n*-hexylmethylamino)-1-naphthaldehyde is substituted for diethylaminobenzaldehyde; mp 132°C. Elemental analysis calculated for $C_{23}H_{26}N_2O_3S_2$: Calculated: C, 62.42; H, 5.92; N, 6.33. Found: C, 62.65; H, 5.99; N, 6.12.

The following additional invention compounds (Examples 29-71) were prepared according to the general procedures described above:

Example 29

(Z) [4-Oxo-5-(4-pyrrolidin-1-yl-benzylidene)-2-thioxo-thiazolidin-3-yl]-acetic acid, mp 266-268°C, MS 349 (M^+).

Example 30

(Z) {5-[4-(4-Butyl-piperazin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid, mp 273°C, MS 420 (M^+).

Example 31

(Z) (4-Oxo-5-{4-[4-(3-phenylpropyl)piperidine-1-yl]-benzylidene}-2-thioxo-thiazolidin-3-yl)-acetic acid, mp 158-159°C, MS 481 (M^+).

Example 32

(Z) {5-[4-(Octahydro-isoquinolin-2-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid, mp 247-248°C, MS 417 (M^+).

Example 33

(Z) {5-[4-(3-Aza-spiro[5.5]undec-3-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propionic acid, mp 258°C, MS 445 (M^+).

Example 34

(Z) 3-[4-Oxo-5-(4-perhydro-azepin-1-yl-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-propionic acid, mp 233°C, MS 391 (M^+).

Example 35

(Z) 4-{5-[4-(3-Aza-spiro[5.5]undec-3-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-butyric acid, mp 180°C, MS 459 (M^+).

Example 36

(Z) {4-Oxo-5-[4-(4-propyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid, mp 260°C, MS 405 (M^+).

Example 37

(Z) 3-{4-Oxo-5-[4-(4-propyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propionic acid, mp 236°C, MS 419 (M^+).

Example 38

(Z) {4-Oxo-5-[4-(4-propyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-butyric acid, mp 181°C, MS 433 (M^+).

Example 39

(Z) [5-(1-Butyl-1,2,3,4-tetrahydro-quinolin-6-ylmethylene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid

Step A:

A mixture of quinoline (3.23 g, 25 mmol) and n-butyl bromide (4.11 g, 30 mmol) are heated to 100°C for 13 hours. Toluene (10 mL) is added to the resulting purple solid. This solid is triturated to a coarse powder, collected, and washed with additional toluene to give 4.89 g of the quinolinium salt.

Step B:

To a solution of this salt (4.89 g, 18.2 mmol) in EtOH (50 mL) is added platinum oxide (240 mg) and stirred under 1 atm of H_2 for 5 hours. The catalyst is removed by filtration through Celite and washed with EtOH. The filtrate is concentrated and the residue dissolved in 0.3N HCI. The aqueous solution is washed with EtOAc, neutralized with 1N NaOH, and extracted with EtOAc. The organic extract is washed with brine, dried over $MgSO_4$ and concentrated to give 2.7 g of 1-butyl-1,2,3,4-tetrahydro-quinoline as a slightly colored oil.

Step C:

Phosphorus oxychloride (1.57 mL, 16.8 mmol) is added dropwise to DMF (2.0 mL), and the mixture is stirred at room temperature for 15 minutes. To this mixture is added dropwise 1-butyl-1,2,3,4-tetrahydro-quinoline (2.65 g, 14 mmol) in DMF (2.3 mL) over a period of 10 minutes. After the initial exothermic reaction ceases, the mixture is heated to 60°C for 20 minutes then poured onto ice (50 g). To the mixture is added 1N NaOH (120 mL), and the resulting heterogeneous mixture is stirred vigorously for 10 minutes and extracted with EtOAc. The organic layer is washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The resulting oil is purified by medium pressure liquid chromatography (MPLC) on silica gel eluting with 5% ethyl acetate/hexane to give 2.61 g of 1-butyl-1,2,3,4-tetrahydro-quinoline-6-carboxaldehyde as a yellow oil.

Step D:

1-Butyl-1,2,3,4-tetrahydro-quinoline-6-carboxaldehyde is reacted with rhodanine-3-acetic acid as previously described to obtain Example 39 as a purple solid; mp 222-224°C, MS 391 (M^+).

Example 40

(Z) 3-{5-[(4aS,8aR)-4-(Octahydro-isoquinolin-2-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propionic acid, mp 237°C, MS 431 (M^+).

Example 41

(Z) 4-{5-[(4aS,8aR)-4-(Octahydro-isoquinolin-2-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-butyric acid, mp 186°C, MS 445 (M^+).

Example 42

(Z) [4-Oxo-5-(4-piperidin-1-yl-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]acetic acid, mp 243°C, MS 363 (M^+).

Example 43

(Z) {5-[(4aS,8aS)-4-(Octahydro-isoquinolin-2-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propionic acid, mp 215°C, MS 431 (M^+).

Example 44

(Z) 4-[4-Oxo-5-(4-perhydro-azepin-1-yl-benzylidene)-2-thioxo-thiazolidin-3-yl]-butyric acid, mp 185°C, MS 405 (M^+).

Example 45

(Z) 4-{5-[(4aS,8aS)-4-(Octahydro-isoquinolin-2-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-butyric acid, mp 173°C, MS 445 (M^+).

Example 46

(Z) 3-[4-Oxo-5-(4-piperidine-1-yl-benzylidene)-2-thioxo-thiazolidin-3-yl]propionic acid, mp 252°C, MS 377 (M^+).

Example 47

(Z) 4-[4-Oxo-5-(4-piperidine-1-yl-benzylidene)-2-thioxo-thiazolidin-3-yl]butyric acid, mp 181°C, MS 391 (M^+).

Example 48

(Z) [4-Oxo-5-(4-azocan-1-yl)-benzylidene-2-thioxo-thiazolidin-3-yl]-acetic acid, mp 274°C, MS 391 (M^+).

Example 49

(Z) {5-[4-(4-Ethyl-4-methyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid, mp 258°C, MS 405 (M^+).

Example 50

(Z) 3-{5-[4-(4-Ethyl-4-methyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propionic acid, mp 237°C, MS 419 (M^+).

Example 51

(Z) {5-[4-(4-Cyclohexylmethyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid, mp 225°C, MS 459 (M^+).

Example 52

(Z) [5-(1-Butyl-2,3-dihydro-1H-indol-5-ylmethylene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid

Step A:

Phosphorus oxychloride (0.69 mL, 7.14 mmol) is added dropwise to DMF (1.09 mL), and the mixture is stirred at room temperature for 15 minutes. To this mixture is added dropwise 1-butylindoline (1.18 g, 6.74 mmol) in DMF (1.0 mL) over a period of 10 minutes. After the initial exothermic reaction ceases, the mixture is heated to 60°C for 20 minutes then poured onto ice. To the mixture is added 1N NaOH (80 mL), and the resulting heterogeneous mixture is stirred vigorously for 10 minutes and extracted with EtOAc. The organic layer is washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The resulting oil is purified by medium pressure liquid chromatography (MPLC) on silica gel eluting with 5% ethyl acetate/hexane to give 0.792 g of 1-butylindoline-5-carboxaldehyde as a yellow oil.

Step B:

1-Butylindoline-5-carboxaldehyde is reacted with rhodanine-3-acetic acid as previously described to obtain Example 52 as a solid; mp >250°C, MS 377 (M^+).

Example 53

(Z) 4-{5-[4-(4-Ethyl-4-methyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-butyric acid, mp 162°C, MS 433 (M^+).

Example 54

(Z) 3-{5-[4-(4-Cyclohexylmethyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propionic acid, mp 205°C, MS 473 (M^+).

Example 55

(Z) 3-{5-[4-(4-Benzyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propionic acid, mp 202°C, MS 467 (M^+).

Example 56

(Z) {5-[4-(4-Benzyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid, mp 221°C, MS 453 (M^+).

Example 57

(Z) 4-[4-Oxo-5-(4-azocan-1-yl-benzylidene)-2-thioxo-thiozolidin-3-yl]butyric acid, mp 199°C, MS 419 (M^+).

Example 58

(Z) 4-{5-[4-(4-Benzyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-butyric acid, mp 147°C, MS 481 (M^+).

Example 59

(Z) 4-{5-[4-(4-Cyclohexylmethyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-butyric acid, mp 168°C, MS 487 (M^+).

Example 60

(Z) 3-[4-Oxo-5-(4-azocan-1-yl-benzylidene)-2-thioxo-thiazolidin-3-yl]propionic acid, mp 224°C, MS 405 (M^+).

Example 61

(Z) 3-[5-(1-Butyl-1,2,3,4-tetrahydro-quinolin-6-ylmethylene)-4-oxo-2-thioxo-thiazolidin-3-yl]-propionic acid, mp 214-215°C, MS 405 (M^+).

Example 62

(Z) 4-[5-(1-Butyl-1,2,3,4-tetrahydro-quinolin-6-ylmethylene)-4-oxo-2-thioxo-thiazolidin-3-yl]-butyric acid, mp 152-154°C, MS 419 (M^+).

Example 63

(Z) {5-[4-(4-Hexyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid, mp 180°C, MS 447 (M^+).

Example 64

(Z) 3-{5-[4-(4-Hexyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propionic acid, mp 190-191°C, MS 461 (M^+).

Example 65

(Z) 4-{5-[4-(4-Hexyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-butyric acid, mp 192-193°C, MS 475 (M^+).

Example 66

(Z) {5-[4-(4-Butyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid

Example 67

(Z) 3-{5-[4-(4-Butyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propionic acid

Example 68

(Z) 4-{5-[4-(4-Butyl-piperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-butyric acid

Example 69

(Z) {5-[4-(4-Pentyl-pyrrolidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetic acid, mp 221°C, MS 419 (M^+).

Example 70

(Z) 3-{5-[4-(4-Pentyl-pyrrolidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propionic acid, mp 222°C, MS 433 (M^+).

Example 71

(Z) 4-{5-[4-(4-Pentyl-pyrrolidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidin-3-yl}-butyric acid, mp 171°C, MS 447 (M^+).

BIOLOGICAL EXAMPLES

Invention compounds of Formula I have been evaluated in several standard in vitro and in vivo assays which are well-established as indicative of clinical usefulness in treating Alzheimer's disease and other conditions associated with amyloid formation.

AMYLOID ASSAYS

BASSR (Beta-Amyloid Self-Seeding Radioassay)

An assay for inhibitors of self-seeded amyloid fibril growth

Materials:

Stock Solutions:

Assay Buffer - 50 mM sodium phosphate, pH 7.5, 100 mM NaCl, 0.02% NaN_3, 1 M urea (filter and store at 4°C)

Soluble Aβ(1-40) peptide (Bachem, Torrance, CA) - 2.2 mg/mL in deionized H_2O (stored in aliquots at -20°C, keep on ice when thawed) will self-seed after 1 week storage. Typically, the solution should be stored until no lag phase is seen in the assay.

^{125}I-labeled Aβ (1-40) - 150K to 350K cpm/μL in 100% acetonitrile- 0.1% trifluoroacetic acid (TFA)-1% β-mercaptoethanol (aliquots stored at -20°C). ^{125}I-labeled Aβ (1-40) can be made in accordance with the procedure set forth by H. Levine, III in *Neurobiol. Aging,* **16**:755 (1995), which is hereby incorporated by reference, or this reagent may be purchased from Amersham, Arlington Heights, Illinois.

Final assay conditions: 30 μM soluble A*β*(1-40) in deionized water in assay buffer + 20K to 50K cpm ^{125}I-labeled A*β*(1-40) per assay. Compound to be tested is dissolved in dimethylsulfoxide (DMSO), typically 5 to 50 mM stock, such that the final concentration of DMSO is <1% v/v in the assay.

Assay: Reaction mixture for 50 assays (on ice) is comprised of 0.1-0.2 μL of ^{125}I-labeled A*^{125}I-labeled Aβ*(1- 40) + 1 μL of soluble *Aβ*(1-40) + 13.5 μL assay buffer per assay. The following are the amounts of the components of the reaction mixture sufficient for 50 assay wells.

5-10 μL ^{125}I-labeled *Aβ*(1-40) dried down
675 μL assay buffer
50 μL soluble *Aβ*(1-40)

Assay Method

1) Prepare reaction mixture above by mixing components and storing on ice.
2) Pipet 14.5 μL of reaction mixture into each of 50 wells on a polypropylene U-bottom 96-well microtiter plate on ice. (Costar 3794).
3) Add 1.7 μL of diluted compound to be tested to each well in a column of eight, including solvent control. Serial 3-fold dilutions from 1 mM (100 μM final) in assay buffer-urea = 7 dilutions + zero. Each 96-well plate can therefore accommodate 11 samples + 1 Congo Red control (0.039-5 μM final in 2-fold steps).
4) Seal the plate with aluminum film (Beckman 538619) and incubate for 10 minutes on ice.
5) Raise the temperature to 37°C and incubate for 3 to 5 hours (depending on the lot of the peptide).
6) Remove the aluminum film and add 200 μL/well of ice cold assay buffer with urea, collecting the radiolabeled fibrils by vacuum filtration through 0.2 μm pore size GVWP filters in 96-well plates (Millipore MAGV N22, Bedford, MA). Determine the radioactivity of the filters using standard methods well-known to those skilled in the art.

BASST (Beta-Amyloid Self-seeding, ThioflavinT)

An assay for inhibitors of self-seeded amyloid fibril growth

METHODS:

Materials:

Stock Solutions:

Assay Buffer - 50 mM sodium phosphate, pH 7.5, 100 mM NaCl, 0.02% NaN_3, 1 M urea (filter and store at 4°C)

Soluble Aβ (1-40) - 2.2 mg/mL in deionized H_2O (store in aliquots at -20°C, keep on ice when thawed) will self seed after 1 week storage. Typically, the solution should be stored until no lag phase is seen in the assay.

Final assay conditions: 30μM soluble *Aβ* (1-40) in deionized water in assay buffer. Compound to be tested is dissolved in DMSO, typically 5 to 50 mM stock, such that the final concentration of DMSO is <1% v/v in the assay.

Assay: Reaction mixture for 50 assays (on ice) comprised of 1 μL of soluble *Aβ* (1-40) + 13.5 μL assay buffer per assay. The following are the amounts of the components of the reaction mixture that result in each of the 50 assay wells.

50 μL soluble *Aβ* (1-40)
675 μL assay buffer

Assay Method

1) Prepare the reaction mix above by mixing the components and storing on ice.
2) Pipet 14.5 μL of reaction mixture into each of 50 wells of a polystyrene U-bottom 96-well microtiter plate (Corning 25881-96) on ice.
3) Add 1.7 μL of diluted compound to be tested to each well in a column of eight, including solvent control. Serial 3-fold dilutions from 1 mM (100 μM final) in assay buffer-urea = 7 dilutions + zero. Each 96-well plate can therefore accommodate 11 samples + 1 Congo Red control (0.039-5 μM final in 2-fold steps).

4) Seal the plate with aluminum film and incubate for 10 minutes on ice.

5) Raise the temperature to 37°C and incubate for 3 to 5 hours (depends on the lot of the peptide).

6) Remove the aluminum film and add 250 µL/well of 5 µM thioflavin T (ThT) [T-3516, Sigma-Aldrich] in 50 mM glycine-NaOH, pH 8.5. Read fluorescence on a plate reader (ex = 440 nm/20 nm ; em = 485 nm/20 nm) within 5 minutes.

BAPA (Beta-Amyloid Peptide Aggregation)

This assay is used to provide a measure of inhibition by a compound against the aggregation behavior of the beta amyloid peptide.

The purpose of this assays is to provide a higher volume method of assaying the amount of beta amyloid aggregation using an endpoint assay based on filtration. In this assay, hexafluoroisopropanol (HFIP) is used to break down the initial amyloid peptide to a monomer state and use a concentration of 33 µM which is high enough so that aggregation will occur at pH 6.0 in several hours.

METHODS:

β-Amyloid Peptide Aggregation, pH 6.0 (BAPA)

In a 96-well plate (Costar 3794), we add 25 µL 50 mM phosphate buffer, pH 6.0, 10 µL 0.5 mg/mL Aβ (1-40) peptide in 20% HFIP + 0.1 µL/assay radioiodinated ^{125}I Aβ (1-40) [^{125}I Aβ(1-40)], and 1 µL of the compound to be tested starting at 50 mM with a concentration of DMSO <1%. Then, we incubate for 2 to 4 hours at room temperature. We stop the reaction with 200 µL of 50 mM phosphate buffer, pH 6.0, and filter it through a 0.2 µm 96-well filter plate (Millipore MAGU N22). We wash the filter plate with 100 µL of the same phosphate buffer. Aggregation was detected on a Microbeta counter after impregnating the filters with Meltilex (1450-441) and is corrected for background.

BATYM ASSAY

METHODS:

Required Aβ (1-42) (California Peptide) was dried from its hexafluoroisopropanol (HFIP) stock solution. The Aβ (1-42) was dissolved in dimethylsulfoxide (DMSO) and then mixed with phosphate buffered saline (PBS) (pH 7.4). The mixed Aβ (1-42) solution was filtered with a 0.2 μm Omnipore membrane syringe filter (Millipore, Bedford, MA). The compound to be tested in DMSO (50 times concentrate) was put into each well (0.5 μL/well) of a 96-well plate. The Aβ (1-42) solution was added into each well (24.5 μL/well). The plate was centrifuged at 1,000 g for 5 minutes and incubated at 37°C for 1 day (Aβ1-42; final concentration 100 μM).

After incubation, Thioflavin T (ThT) (30 μM) solution in glycine-NaOH buffer (pH 8.5, 50 mM) was added into each well (250 μL/well), fluorescence was measured (ex. 440/20 nm, em; 485/20 nm) using a fluorescence plate reader. The inhibitory activity was calculated as the reduction of fluorescence with the following formula:

$$\text{Inhibition (\%)} = \{(F(A\beta)-F(A\beta+\text{compound})\}/\{F(A\beta)-F(\text{solvent}+\text{compound})\} \times 100 .$$

The IC_{50}'s were calculated by a curve fitting program using the following equation. The data were obtained from two different experiments in triplicate.

$$\text{Inhibition}(x) = 100-100/\{1+(x/IC_{50})^n\}.$$

x = concentration of tested compound (M).

IC_{50} = (M).

n = Hill coefficient.

Representative compounds of Formula I have exhibited inhibitory activities (IC_{50}) ranging from about 0.1 μM to greater than 100 μM in the foregoing assays. The results of these assays for specific representative compounds of the present invention are shown in the table below.

Amyloid Inhibition

Example No.	BASST IC_{50} µM	BAYTM IC_{50} µM	BASSR IC_{50} µM	BAPA IC_{50} µM
1	12.4	31.4	65.3	11.3
2	1.5	3.95	36	>60
3	3		41	15
4	2		31	12
5	2	2.17	7	8
6	3	2.35	10	13.5
7	8	12.4	10	74
8	1.5	1.63	12	56
9	1.2	1.73	8	37
10	4.2	3.98	3	71
11	7	9.72	17.5	23.5
12	6	4.09	31	9.5
13	3		62.5	17.7
14	1	3.13	10	7.5
15	33.7	3.33	11.4	44
16	5		33.5	56
17	5	9.46	15	53
18	0.2	2.58	13	43
19	18		10	39
20	0.4	1.6	4.5	33
21	0.2	3.1	11	14
22	2	2.03	7.5	7
23	2	2.64	2	4
24	1.8	1.95	5	3
25	6	3.72	10.5	
26	1	2.30	3	
27	30	8.46	20	
28	1	1.61	25	33
29	10, 6		>100	>60
30	30	>100	>100	11, 8
31	0.3		80	51
32	1, 0.9	2.84	8, 2	>60, >60
33	0.6	2.63	2.2	33, >60
34	1	6.18	10	>60, 63
35	0.2	1.88	1.8	>60, >100

Amyloid Inhibition (cont'd)

Example No.	BASST IC_{50} µM	BAYTM IC_{50} µM	BASSR IC_{50} µM	BAPA IC_{50} µM
36	1.1	3.99	0.7	59, >60
37	0.4	2.74	3	55, 79
38	0.2	3.17	1	>60, 69
39	2	5.05	12	101
40	0.4, 0.8	3.81	52, 55, 30	98
41	0.5, 0.3	4.03	12, 18, 11	50
42	8	26.5	>100	5
43	0.3	2.28	18, 15	7
44	0.7	5.45	19, 20	6
45	0.3	1.87	10, 8	7
46	3	13.9	>100	5
47	1.8	8.02	75, 60	12
48	1	7.64	12	8
49	1.2	4.36	60	7
50	0.2	4.52	19	80
51	0.2	2.56	15	71
52	3	11.5	>100	30
53	0.3	3.21	>100	40
54	2	2.76	75	>60, >100
55	0.7, 0.2	2.87	15, 8	>60
56	0.1	4.31	>100	38
57	0.3	5.17	12	40
58	0.2	3.02	6	63
59	0.2	3.23	4.2	88
60	0.6	7.27	70	72
61	1.5	4.22	4.8	30
62	1	5.08	3.5	60
63	0.5	2.64	25	>60
64	0.6	2.84	21	>60
65	0.3	2.21	>100	>60

The invention compounds have also shown good activity in standard in vivo mouse assays commonly used to evaluate agents to treat diseases related to aggregation of amyloid proteins, especially Alzheimer's disease. Such assays are

described by Axelrod et al., *Lab. Invest.* 1982;47(2):139-146; and by Stenstad et al., *J. Biochem.* 1994;303(Pt 2):663-670. In one assay, amyloid protein is induced into the spleen of mice by subcutaneous injections of silver nitrate, Freund's complete adjuvant, and an intravenous injection of amyloid enhancing factor. Silver nitrate is administered each day through Day 11. Test compounds are administered to the mice daily starting on Day 1 through Day 11. On Day 12, the animals are sacrificed, and the spleens are removed, histologically prepared, stained with Congo red, and the percent area of the spleen occupied by birefringent, Congo red - stained amyloid is quantitated microscopically. Invention compounds evaluated in this test have shown good inhibition of splenic amyloid deposition relative to untreated controls.

Another in vivo assay in which the invention compounds have been evaluated uses transgenic mice. The mice bear a human β-amyloid precursor protein transgene with a prion promoter, as descried by Hsiao et al, "Correlative Memory Deficits, Aβ Elevation, and Amyloid Plaques in Transgenic Mice," *Science* 1996;274:99-102. These transgenic mice develop β-amyloid deposits at about 9 months of age. By 15 months, diffuse and compact senile plaques are abundant, primarily in the neocortex, olfactory bulb, and hippocampus. Invention compounds are administered orally to the mice beginning at the age of 8 months (just prior to the onset of amyloid deposits) and continuing for several months (up to about age 14-18 months). The animals are then sacrificed, and the brains are removed. The amount of amyloid in the brain is quantitated both histologically and biochemically. Invention compounds evaluated in this model have shown good inhibition of amyloid accumulation in the cortex and hippocampus relative to untreated controls.

The foregoing data establish that invention compounds of Formula I are potent inhibitors of protein aggregation, and are thus useful in treating diseases associated with amyloid deposits and to image amyloid deposits for diagnostic use. The compounds typically will be used in the form of pharmaceutical formulations for therapeutic use, and the following examples further illustrate typical compositions.

Example 72

Tablet Formulation

Ingredient	Amount
Compound of Example 1	50 mg
Lactose	80mg
Cornstarch (for mix)	10 mg
Cornstarch (for paste)	8 mg
Magnesium Stearate (1%)	2 mg
	150 mg

The compound of Example 1 is mixed with the lactose and cornstarch (for mix) and blended to uniformity to a powder. The cornstarch (for paste) is suspended in 6 mL of water and heated with stirring to form a paste. The paste is added to the mixed powder, and the mixture is granulated. The wet granules are passed through a No. 8 hard screen and dried at 50°C. The mixture is lubricated with 1% magnesium stearate and compressed into a tablet. The tablets are administered to a patient at the rate of 1 to 4 each day for prevention of amyloid and treatment of Alzheimer's disease.

Example 73

Parenteral Solution

In a solution of 700 mL of propylene glycol and 200 mL of water for injection is added 20.0 g of the compound of Example 26. The mixture is stirred and the pH is adjusted to 5.5 with hydrochloric acid. The volume is adjusted to 1000 mL with water for injection. The solution is sterilized, filled into 5.0 mL ampoules, each containing 2.0 mL (40 mg of Example 26), and sealed under nitrogen. The 10 solution is administered by injection to a patient suffering from medullary carcinoma of the thyroid and in need of treatment.

Example 74

Patch Formulation

Ten milligrams of (Z) 4-{5-[4-(4-benzylpiperidin-1-yl)-benzylidene]-4-oxo-2-thioxo-thiazolidine-3-yl}butyric acid is mixed with 1 mL of propylene

glycol and 2 mg of acrylic-based polymer adhesive containing a resinous cross-linking agent. The mixture is applied to an impermeable backing (30 cm^2) and applied to the upper back of a patient for sustained release treatment of amyloid polyneuropathy.

The invention and the manner and process of making and using it are now described in such full, clear, concise, and exact terms as to enable any person killed in the art to which it pertains, to make and use the same. It is to be understood that the foregoing describes preferred embodiments of the present invention and that modifications may be made therein without departing from the spirit or scope of the present invention as set forth in the claims. To particularly point out and distinctly claim the subject matter regarded as invention, the following claims conclude this specification.